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Effect of Ammonia on Post-diapause Western Corn Rootworm Eggs and Larvae

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EFFECT OF AMMONIA ON POST-DIAPAUSE WESTERN CORN ROOTWORM EGGS AND LARVAE

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EFFECT OF AMMONIA ON POST-DIAPAUSE

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Corn rootworms (Coleoptera, Chrysomelidae) are the major economic insect pests of corn produced in the Midwest. Economic loss to the grower is through yield reduction caused by larval and adult damage and the cost of insecticidal control measures. Larval damage to corn roots is by tunneling in the roots and damage to the cortical parenchyma. Most of this damage occurs in late June and in early July. Brace root feeding by the larvae can cause the plant to lodge. Adults also damage corn by feeding on all above ground portions with primary damage to silks and tassels. This feeding disrupts pollination resulting in improper filling of ears and subsequent yield reduction.

Three species of corn rootworms are present in South Dakota: the northern corn rootworm (NCR), *Diabrotica longicornis barberi* Smith and Lawrence; the western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte; and the southern corn rootworm (SCR), *Diabrotica undecimpunctada howardi* Barber. The SCR is not a significant pest of corn in South Dakota as it is unable to overwinter and migrating adult populations are generally low until late in the season. The NCR and the WCR have been major problems the past two decades. The WCR causes the majority of the damage in continuous cornfields and the NCR in continuous and first year corn.

Northern corn rootworms were first recognized as being damaging to corn in 1880 by Charles Riley (Hill et al. 1948). The WCR were found to damage continuous corn in Colorado in 1909 (Gillette 1912). Tate and Bare (1946) reported the WCR in Nebraska in 1929. Economic populations of the WCR were not common in Nebraska until 1961 (Ball and Weekman 1962). Rootworm resistance coincided with the spread of WCR from Nebraska to Ohio.

Gravid adult WCR and NCR females lay their eggs in the soil of cornfields during late summer and fall. A minority of the eggs are deposited in fields containing crops other than corn or in ditches where weeds and flowering plants are present (Branson and Ortman 1970). Rootworms overwinter as diapausing eggs and hatch in early June. Pupation begins in late June and early July. Adult emergence begins about 10 to 14 days thereafter. Emergence continues throughout July and August and into early September.

The insect targeted in the laboratory and in artificial infestations was the WCR and both WCR and NCR in the field associated with continuous corn production.

The objective of this research was to determine if ammonia affected mortality of the WCR in the laboratory, and the effect of rates and placement of aqua ammonia and anhydrous ammonia on survival of post diapause WCR eggs in the soil in the field.

LITERATURE REVIEW

Western corn rootworms overwinter as eggs in the soil with oviposition occurring during August. Ball (1957) studied the egg-laying habits of the WCR and found that 80% of the eggs were in the upper 15.24 cm, 58% in the top 10.16 cm and 23% in the top 5.08 cm. Patel and Apple (1967) reported that eggs were found in decreasing numbers at 10.16, 20.32 and 25.40 centimeters from the row and 88% of the eggs were observed in the top 7.62 cm of soil. Foster et al. (1979) found 85% of NCR eggs in the top 10 cm of soil. Gustin (1979) also reported that the majority of eggs were found in the top 15 cm of soil.

Soil temperature, soil moisture and soil condition are important variables in determination of hatch and development of corn rootworm eggs. Soil temperature appears to be the major factor influencing time of hatch of eggs in the spring.

Chiang and Sisson (1968) estimated the threshold temperature at 11.1°C for egg development of the NCR. The thermal constant for first hatch was 400 degree-days above 11.1°C. Patel and Apple (1967) estimated the threshold temperature for the NCR in Wisconsin to be 9.7°C and later changed it to 11.1°C (Apple et al. 1971). Wilde (1971) estimated the threshold temperature for WCR in Minnesota to be 11.1°C and 379.5 degree days above 11.1°C for first hatch to occur.

A four year study in Missouri showed that WCR eggs began hatching about June 1, and proceeded at a rate of 2.91% per day until hatch was completed (Musick and Fairchild 1971). Wilde et al. (1972) found that fewer days were required for the initial hatch of 50% of the

WCR eggs from South Dakota or Minnesota than from Iowa, Kansas, Missouri, or Nebraska.

George and Ortman (1965) demonstrated that diapause does occur in the egg of the WCR and chilling is required for hatch to occur. Beck (1968) defined diapause as a "genetically determined state of suppressed development". Patel and Apple (1967) found that hatch begins after diapause is broken by low temperature and that egg survival is reduced when temperatures reach -2°C or below for 2 weeks. Krysan (1978) observed that WCR eggs enter diapause in the fall and diapause lasts through the onset of cold temperatures. Gustin (1980) proposed that egg mortality due to soil temperature could occur in two ways: by winter soil temperatures dropping below the WCR thermal deathpoint, or by autumn soil temperature above the threshold for development. Gustin found that soil temperatures in eastern South Dakota insulated the eggs from intermittent warming spells with post-diapause development inhibited by temperatures until spring. Branson et al. (1975) treated WCR eggs to a chill period of 5°C for 120 days then incubated the eggs at 20° to 25°C until hatch. Prediapause eggs developed to the diapause stage either before or during chilling and the eggs remain quiescent during chilling. Post-chill eggs develop rapidly until hatch. The result of chill is synchronization of hatch.

Patel and Apple (1967) reported that under laboratory conditions, 13% of the northern corn rootworm eggs hatched in the absence of a chill period. Chiang (1973) indicated that exposure to cold temperatures in order to stimulate hatching can be continuous for up to two winters. One explanation is that eggs placed deep in the soil did not receive enough heat units the first year.

Kirk et al. (1968) found that gravid WCR females had an oviposition preference to soil particles larger than 1 millimeter in size. Laboratory tests were conducted to determine WCR larval survival in relation to soil texture by Turpin and Peters (1971). Their work indicated that newly hatched larvae in petri dishes moved from sand to clay but not from clay to sand. The sandy soil has less water holding capacity than a finer textured soil such as a loam or a clay. Larvae are suspected to suffer from abrasion by sand particles in the soil and from subsequent dessication when exposed to air.

Gustin (1979) reported that when gravid WCR females were given a choice in the laboratory between high and low moisture soils, significantly more eggs were oviposited in soils with high moisture levels. Kirk (1975) found that corn plants direct rainfall to the base of the plant because of the shape of their leaves. This extra moisture makes soil cracks at the base of the plant more attractive for oviposition. He later found that eggs were not deposited in cracks unless the beetles had access to a moist soil surface (Kirk 1979).

Sechriest (1969) studied the biology and behavior of corn rootworms in Illinois during 1968. He observed that over 90% of the larvae are found within 10.16 cm of the plant base and in the upper 10.16 cm of soil. If corn is planted between the old rows larval survival may be affected. Larvae are capable of migrating in the soil but it has been observed that placing corn rows midway between the rows of the preceding year may reduce larval survival in minimum tillage fields (Chiang et al. 1971). Musick and Collins (1971) reported that larval populations decreased 34% for each 25 cm the new row was planted from the old row.

Chiang (1973) reported that larvae migrate to corn roots and feed primarily on new fine root hairs and outer cortical tissue. Older larvae invade the cortical parenchyma. Channels can be visibly seen in the roots which are caused by larval feeding in the stele.

Yield reductions in fields having similar degrees of infestation may vary from a total loss to no appreciable loss depending on stage of growth, moisture, soil fertility and variety of corn used (Tate and Bare 1946). If favorable conditions exist, the corn plant can compensate for damaged root systems. Harvesting can be difficult because of lodging of the damaged plants.

Damage to roots from larval feeding can be assessed indirectly by the force needed to pull a plant from the soil or by a root rating system. The root rating system is a numerical scale in order of increasing damage developed by Hills and Peters (1971). The economic rating is between 2.5 and 3.0 (Turpin et al. 1972) varying from state to state. Branson et al. (1980) observed that root damage rating was the most sensitive measurement for determining the level of infestations and damage when measuring plant response to corn rootworm infestations. They found that as few as 100 eggs per 30.5 cm of row had a highly significant effect on root damage ratings.

Recent evidence has shown that WCR can complete their immature stages on crops other than corn, including many weed species. Branson and Ortman (1970) found that rootworms were able to complete immature stages on 13 of 18 grasses. Kirk et al. (1968) observed that gravid females were more attracted to weedy patches in fields than to corn trash. Hill and Mayo (1980) observed damage on first year corn where eggs had been deposited in small grain stubble the previous year.

Adult WCR first emerge and appear in fields very early in July in Nebraska (Ball 1957). Short and Hill (1972) collected adults in Nebraska on July 15 and maximum female emergence occurred on August 6. Howe et al. (1963) reported an initial emergence of corn rootworms on August 1 in South Dakota. WCR have appeared in early July in South Dakota (Walgenbach personal communication).

Short and Hill (1972) found a preoviposition period of 20-23 days in east central Nebraska. Branson and Johnson (1973) reported a mean preoviposition period of 14.3 days in South Dakota. Evidence suggests a shorter preoviposition period for areas with shorter growing seasons.

Temperature, photoperiod and moisture affect adult activity and oviposition. Rootworms did not oviposit when the daily minimum temperature was below 10°C in Nebraska (Ball 1957). Mihm and Chiang (1974) observed that oviposition was decreased at temperatures of 5°, 10° and 15°C, but increased and resumed when temperatures of 20° and 25°C returned.

Corn rootworms have a high level of activity between 5 p.m. and 8 a.m. Van Woerkom et al. (1980) found the maximum activity for males and females occurred at 23°-25°C and 25°-27°C, respectively, under laboratory conditions. Male corn rootworms were more active than females, but female activity exceeded male activity at high temperature ranges. Ball (1971) found that rootworms are more active at dusk, a higher mean number of eggs should have been deposited during late afternoon if oviposition is related to activity.

The environment of a cornfield is such that moisture around the plants is present and evaporates as temperatures increase. Ball (1971) observed that the number of eggs that a female deposits is related to moisture. When substrate moisture ranged between 30 and 60%, oviposition was maximum. When substrate moisture content was zero or approached saturation, oviposition decreased.

Kirk et al. (1968) studied ovipositional preferences of the WCR in the laboratory. Beetles preferred large soil particles, soil cracks and moist soil for oviposition sites. Beetles preferred soil particles above 1 mm in size. Clumps of grass such as foxtail were preferred over corn stalks and surface trash. It was concluded that all of these factors combine to influence oviposition patterns in a cornfield. Ball (1957) observed that the female WCR laid eggs near the base of the cornstalk. Patel and Apple (1967) found no NCR eggs between corn rows. Kirk (1979), in field studies in eastern South Dakota, recovered more WCR and NCR eggs from soils containing drought cracks than from soils lacking drought cracks.

Control Measures

Forbes (1883) realized that the NCR survived only on corn which prompted the use of crop rotation as a satisfactory control measure. Resistant corn hybrids are useful (Sifuentes and Painter 1964) in reducing damage, however, Fitzgerald and Ortman (1964) contended that the feasibility of breeding varieties of corn resistant to larval feeding has not been established. Tate and Bare (1946) studied the effect of tillage practices on rootworms. Rootworm damage, as evaluated by lodged corn, was reduced by fall plowing.

In recent years, producers use their land to produce corn annually because of its high cash value and importance on farms for livestock feed. Because of this practice for growing continuous corn, the WCR has become an economic pest necessitating other control measures such as the use of insecticides.

Hill et al. (1948) demonstrated that soil insecticides were effective in controlling rootworms. A reduction of damage to corn was observed with .44 to 1.78 kg of lindane per hectare applied as a pre-plowing broadcast spray.

Resistance to chlorinated hydrocarbon insecticides was first noted in 1959. Ball and Weekman (1962) noted that resistance to aldrin and heptachlor continued and increased in Nebraska in 1960 and 1961. Howe et al. (1963) reported resistant corn rootworm populations in South Dakota in 1962.

South Dakota State University recommendations to growers include rotation of their corn rootworm insecticide each year (Kantack et al. 1975). Crop rotation has been helpful in the past, but damage to first year corn following small grains has been observed since 1975 from the NCR. Large numbers of adult northerns are found in weedy small grain fields in the fall, indicating differences in the biology of the NCR. Therefore, SDSU has recommended the treatment of first year corn in recent years.

The use of organic or inorganic fertilizers as a control for corn rootworms has not been scrutinized carefully. Forbes (1894) stated that certain fertilizers such as potash salts might have some deleterious effect on larvae in the soil. A field study in eastern Nebraska (Hill et al. 1948) found the lowest percentage of lodging caused by rootworm damage and the highest yields occurred in plots receiving 35.6 to 71.0 kg nitrogen fertilizer per hectare at the time of last cultivation. Plots treated with 80-0-0 had 34.5% plants lodged and 2375 kg per ha compared to non-fertilized plots having 81.5% of the plants lodged and 1036 kg per ha. They concluded that nitrogen did not affect the rootworm population, but brought about quick recovery of the plant. Unfortunately, root ratings were not used in 1948. Muma et al. (1949) applied 35.6 kg of nitrogen per hectare in the form of ammonium nitrate in a band below the soil surface on each side of the corn rows. They found that all plots receiving nitrogen gave a significant increase in yield. Plots receiving nitrogen were similar in results to plots receiving 4.5 kg of rotenone or .89 kg of toxaphene per hectare, however, these compounds are not good soil insecticides. At another location in eastern Nebraska, Hill found that less beetles per plant were noticed in plots receiving 35.6 kg actual nitrogen per hectare than check plots or plots receiving .89 and 1.78 kg toxaphene and plots with 4.45 kg rotenone per hectare (Hill et al. 1948).

Control of corn rootworms with starter fertilizer-insecticide combinations was examined by Weekman (1965). He reported that corn rootworms were controlled by liquid starter fertilizer-insecticide combinations in 1964. Apple (1968) studied liquid and dry starter fertilizer-insecticide combinations and found they must be banded on both sides of the row to be effective. In a three year study, Musick (1974) found that the application of 340.5 Kg/ha on 1.02 meter rows of the liquid starter fertilizer Arcadian Poly-N® (10-34-0) mixed with Bux® Or Dyfonate® provided some protection, but the combinations were inconsistent. Of the two insecticides, combinations containing Bux® performed significantly better than Dyfonate® combinations. In the field, applications of the combinations were applied either on one side of the corn row or on both sides. Because of larval hatch less than one week later, the ineffectiveness of the combinations was due to placement at one side or both sides of the row precluded the mixture's movement into the root zone. Mayo (1977) reported that when starter fertilizer-insecticide mixtures are placed at seed depth they resulted in significantly less root damage than in untreated rows. The liquid fertilizer was 10-36-0 applied at 89 kg per hectare of carbofuran, ethoprop, fonofos, fensulfothion, Ga 12223, or SRA 12869.

The use of ammonium nitrate at different rates to control of damage from corn rootworms has been studied by Olson (1971). Ammonium nitrate at rates from 0 to 178 kg per ha were broadcast in the spring prior to planting. Results indicated that as the rate of nitrogen increased, a slight decrease in root damage ratings occurred, and an inconsistent decrease in numbers of rootworms per plant. Although trends did occur, drawing conclusions were difficult because of extremely dry field conditions.

Few reports relate rates of nitrogen fertilization to insect pest damage. Goyer and Benjamin (1972) studied the influence of ammonium nitrate on the pine root weevil (Hylobius rhizophagus) in jack pine plantations. Weevil infested stands had less total nitrogen than adjacent fertilized stands. Application of 223 kg per ha of ammonium nitrate (33-0-0) caused a drastic reduction in larval feeding and development which resulted in mortality. Lack of nitrogen appeared to be related to larval feeding responses as larvae fed more on roots from the weevil infested area. Haseman (1946) observed that each insect species had its own nutritional requirements and that an insect could survive better on crops grown on a nutrient deficient soil than in a nutrient sufficient soil. Fertilization might discourage or influence species which prefer infertile soils, Haseman (1950) found that European corn borers (Ostrinia nubialis) survived better on corn grown on fertile soils.

Cannon and Ortega (1966) studied European corn borer larvae infesting susceptible and resistant corn varieties treated with several rates of nitrogen. Survival of first and second brood larvae was 10 times greater at 200 ppm than at 100 ppm. Hogan (1961) found that ammonia compounds were effective in accelerating the rate of termination of diapause in eggs of the field cricket (Acheta commodus).

Control measures consisting of the application of ammonium nitrate, or starter fertilizers on reduction in damage caused by corn rootworms has been studied to a limited extent. No reports of research were found on anhydrous ammonia and its effect on corn rootworms.

Moraghan (1980) reported that anhydrous ammonia is the major form of nitrogen fertilizer in the U.S. Anhydrous ammonia has a great affinity for clay particles in the soil and is soluble in water (up to 44% by weight) (Hedman and Turner 1954). The boiling point of NH_3 under atmospheric pressure as injected into the soil is -33°C (Lyons 1966). At soil temperatures between 4.4°C and 10°C or lower, the nitrogen remains as an ammonium ion and at higher temperatures it converts to nitrite, then to nitrate.

Bagchi et al. (1977) reported that reactions of anhydrous ammonia in soil are different from aqua ammonia or ammonium ions related to dissociation of ammonium salts. They found that ammonia moves very little in soil when injected. The immediate effect of injection in soil was to create an alkaline zone at the point of injection and immediate vicinity which caused partial sterilization of the soil and reduction in the number of nitrifying organisms. They also found an increase in available nitrogen which was accompanied by immobilization and then gradual mobilization. Dead microbial cells were noted in samples following the application of ammonia. Ghosh (1977) observed that anhydrous ammonia enters the soil in a gaseous state from the applicator. After injection some of the ammonia is retained in a localized, horizontal, cylinder-shaped zone with a diameter of 5-12 cm depending on application rate, soil texture, cation exchange capacity, soil organic matter, soil moisture and bulk density of the soil. The rest of the ammonia dissolves in soil water and forms ammonium hydroxide. Ghosh (1977) concluded that if the moisture content of the soil was more than field capacity the movement of ammonia was restricted resulting in the loss of gaseous ammonia.

Careful placement of anhydrous ammonia in the row prior to corn planting or in the fall over the row could be deleterious to rootworm eggs in the soil. Tisdale and Nelson (1975) reported that free ammonia is toxic to living organisms. The ultimate effect of anhydrous ammonia application is to lower the pH of the soil which is accompanied by an initial and marked reduction in the numbers of soil microorganisms. Nitrification begins after injection in the periphery of the zone of the retained ammonia. Nitrification proceeds inward after the toxic effect of the gas subsides.

MATERIALS AND METHODS

Laboratory Methods

The effect of contact aqua ammonia on post-diapause WCR eggs was examined by applying aqua ammonia (17.8-0-0) to the center of petri dishes containing five sheets of moistened filter paper. Thirty, seven day post-diapause WCR eggs were placed in 100 x 200 mm plastic Optilux® petri dishes on top of the moistened filter paper. The post-diapause eggs were obtained from the Northern Grain Insects Research Laboratory (NGIRL), Brookings, South Dakota. The WCR collection and rearing procedures followed by NGIRL included fall collection of adult WCR beetles from cornfields and trap crops of cucurbits during August, 1980. Gravid females were caged, fed, and allowed to oviposit in petri dishes (100 x 200 mm) containing - 80 mesh soil watered to 20% moisture by weight. Eggs collected by NGIRL personnel from cages were exposed to pre-chill temperatures of 22° to 25°C for two weeks. After the pre-chill period, eggs were placed in refrigeration at 5°C for 120 days followed with a post-chill exposure of eggs to 20° to 25°C until hatch. Hatch begins seven to ten days thereafter (Branson et al. 1975). Rates of 0, 89, and 267 kilograms of nitrogen per hectare were applied by pipet corresponding to 0, 1.8, and 5.3 grams of aqua ammonia per petri dish. Each of the three treatments was repeated five times. Eggs remained on the filter paper in a controlled environment of 60% relative humidity and 25° to 28°C until hatch occurred approximately seven to ten days later. Eggs which did not hatch were recorded as dead.

A second laboratory experiment was conducted with rates of aqua ammonia (17.8-0-0) applied to seven-day post-chill WCR eggs in a Kranzburg loam soil. The soil had a pH of 7.5, containing 3.9% organic matter, 113.83 kilograms water soluble nitrate nitrogen per hectare before treatment, bulk density of 1.1628 g per cm^3, 34% sand, 42% silt, and 24% clay. Seventy-five grams of air dry loam soil screened to 80 mesh was placed in 100 x 200 mm plastic Optilux® petri dishes. The soil was moistened to 20% moisture by weight with tap water. Thirty uniform appearing post-diapause WCR eggs were placed in the center of each petri dish containing the moistened soil and stirred carefully with a spatula to insure contact with the soil. Aqua ammonia (17.8-0-0) was applied at rates of 0, 89, 178, 356, 712, and 1415 kilograms of nitrogen per hectare corresponding to 30.3, 60.5, 121.0, 242.0, and 481.1 grams of nitrogen into the petri dish. Rates of nitrogen were calculated using surface area and depth of the petri dishes. Six treatments and ten replicates were arranged in a controlled environment where temperatures of 25°-28°C and relative humidity of 60% were kept constant for fourteen days. Each dish containing eggs was then washed into a 60 mesh sieve and rinsed with tap water so that the eggs were retained on top of the sieve. Eggs were then rinsed into filter papers for examination under a dissecting microscope. Numbers of live and dead eggs were recorded. Dead eggs were those which appeared swollen, crushed, or those which had burst open. Dosage mortality responses were calculated by the methods described by Daum et al. (1962). Representative hatch was determined by placing thirty, seven-day post chill WCR eggs in each of five petri dishes containing moistened filter paper. These eggs were kept at 60% relative humidity and 25°-28°C until hatch to first instar larvae occurred. Percent hatch of the eggs used in the study was determined to be 78%. Petri dishes were prepared in the same manner without WCR eggs for soil test analysis. For seven days, at daily intervals after the application of aqua ammonia, petri dishes were collected and refrigerated for soil tests. Soil was tested for pH, water soluble nitrate-nitrogen using a nitrate electrode (Orion Model 92-07). Organic matter was determined using a potassium dichromate and sulfuric acid solution for digestion, and measured at 645 mu with a Coleman Junior Spectrophometer, Model 6C (Modified Walkley-Black procedure).

A final laboratory experiment was implemented to monitor WCR egg and/or larval mortality to several rates of aqua ammonia in the Kranzburg loam soil. The soil was screened to .65 cm and 1200 cc of the soil were added to 15.24 cm diameter clay pots over plastic saucers. Four hundred ml of tap water were added to the top of the pots the first day to ensure good capillarity and later through the saucers below the pots. The soil ca was maintained at 20% moisture by weight. Three TS-91 Sokota hybrid corn seeds (fungicide-free) were planted per pot and later the seedlings were kept under a 16-hour photoperiod using Agri-lites®. Air temperature was maintained at 25°-28°C and 60% relative humidity. After germination six days later, aqua ammonia was applied one inch deep in the center of each pot.

Rates used were 0, 89, 178, 267, 356, 712, and 1415 kg of nitrogen per ha corresponding to 0, 3.3, 6.5, 10.2, 12.1, 24.2, and 48.1 g of nigrogen. Thirty post-chill WCR eggs were selected for uniformity using a dissecting microscope and were placed on filter papers, then innoculated into the soil seven days after the aqua ammonia application. The eggs were transferred to the soil by washing them from the filter paper into a slight depression in the soil in the center of each pot. The eggs were stirred carefully with a spatula to ensure contact with the soil. The experiment was conducted for 27 days after innoculation of eggs into the soil (Figure 1). The seven treatments and ten replications were analyzed by chi-square. All of the soil in each pot was examined on the 28th and 29th days of the experiment (Figure 2). Numbers of live second and third instar larvae were recorded. The soil from each pot was collected, dried, and analyzed for water soluble nitrate-nitrogen, pH, and soil ammonia using an ammonia electrode after soil was extracted with potassium chloride. Dosage (fertilizer rate) mortality responses were calculated by methods described by Daum et al. (1962).

Field Methods

Five field locations in two counties in eastern South Dakota in 1981 were selected because of high adult corn rootworm survey counts in 1980 (Table 1). All fields were soil sampled on April 28, 1981 for initial N, P, K, and pH values (Table 2). 1980 corn rows were marked at the edge of each field with flags. Seven fertilizer treatments, replicated four times, were arranged in a randomized complete block

Figure 1. Laboratory arrangement of experiment 3.

Figure 2. All soil in 15.24 cm clay pots were examined with a spatula for second and third instar WCR larvae.

Table 1. Experimental location descriptions for 1981.

Location	No. beetles per plant (August 1981)	Soil Texture	% Sand	% Silt	% Clay	County	Field preparation	Method
1	4.7	Clay loam	22.24	40.16	37.60	Kingsbury	Spring	Plow
2	4.7	Clay loam	20.52	41.16	38.32	Kingsbury	Spring	Disked
3	11.6	Silty clay loam	22.32	58.21	19.47	Kingsbury	Spring	Plow
4	5.7	Silt loam	33.72	52.25	14.03	Kingsbury	Spring	Plow
5	6.0	Loam	40.30	37.29	22.41	Brookings	Spring	Plow
6*	0	Loam	45.68	39.44	14.88	Brookings	--	

*Location 6 used for artificial infestation only. Not planted to corn preceding 1981 experiment.

Table 2. 1981 Initial soil test results.*

Location	pH	P (kg/ha)	K (kg/ha)	NO_3-N (kg/ha)	% O.M.	Texture
1	7.0	26	611	7.0	2.4	Clay loam
2	6.8	21	574	8.3	2.8	Clay loam
3	6.2	74	781	13.4	3.0	Silty clay loam
4	6.5	19	316	10.4	2.3	Silt loam
5	6.0	22	344	9.0	2.7	Loam
6	6.0	22	---	250	3.2	Loam

*Soil test results prior to fertilization.

design. Treatments were no fertilizer (0 kg/ha), 89, 178, and 267 kg of N per ha in the form of anhydrous ammonia (82.5-0-0) and 89, 178, and 267 kg nitrogen per ha in the form of aqua ammonia. Plots measured 1.93 m by 15.7 m.Anhydrous ammonia was knifed into the marked corn rows and aqua ammonia was broadcast sprayed using a bicycle sprayer onto the soil surface of designated plots at location 1 on May 7, 1981. On May 11, 1981, locations 2, 3, and 4 were treated with aqua ammonia and immediately disked. Locations 3 and 4 were treated with anhydrous ammonia on May 11. On May 12, location 5 had both fertilizer substances applied and plots receiving aqua ammonia were disked following application.

Anhydrous ammonia was applied as close to 1980 rows as possible using an anhydrous ammonia applicator with a regulator and two knives spaced 96 cm apart attached to a Ford 3000 tractor (Figure 3). All hoses on the applicator were cut to the same length to ensure uniformity of ammonia flow rates. Anhydrous ammonia treatments were calibrated using tractor speed and settings on the regulator. Weights of the NH_3 tank were checked before and after soil application.

Corn was planted at locations 1, 2, 3, and 4 on May 12, 1981 using Pioneer 3980. Lasso was banded at planting time at three pounds A. I. per acre. Location 5 was planted on May 26, 1981. Corn was planted as close to the anhydrous ammonia placement as possible in .96 meter rows. Knife marks were visible at all five locations.

Soil samples were taken at all locations, 15 cm deep, one week after planting. These samples were not thoroughly dried and

Figure 3. Anhydrous ammonia application set-up with two row knife injector.

accurate analysis was not possible so the data were not used. Three weeks after planting, all plots were again sampled at the 15 cm depth and analyzed for pH, water soluble nitrates, phosphorus, potassium (using 1 N ammonium acetate as extractant and examination of filtrates with a Perkin-Elmer flame photometer, Model 146), and ammonia-nitrogen. Analysis of soil ammonia involved soil extraction with potassium chloride and steam distillation with magnesium oxide. The distillate was evaluated immediately using a Coleman Junior Spectrophotometer, Model 6C. Samples were initially examined with wavelength of 415 mu but changed because of unreliable values when determining the standard curve. Samples were repeated and evaluated at 425 mu according to procedures outlined by Metson (1961).

A sixth location in Brookings County was artificially infested with post-chill WCR eggs obtained from the NGIRL. This experiment was conducted on a loam soil which was planted to grass and garden for several years preceding the experiment. Soil test results and Bouyoucous hydrometer texture are reported in Tables 1 and 2. Cages were fashioned from bottomless 20 l plastic pails. Forty-eight cages were arranged in a randomized complete block design with four treatments and four replicates. The cages were pressed into the plowed and disked soil until 5.08 to 7.62 cm remained above the soil surface (Figure 4). Aqua ammonia was applied at rates of 0, 89, 178, and 267 kg of nitrogen per ha corresponding to 0, 4, 8, and 12 ml of aqua ammonia into a small indentation in the center of the cage. Three Sokota TS-91 fungicide-free corn seeds were planted one

Figure 4. Bottom portion of adult trap with rim exposed above soil surface.

week after aqua ammonia application and thinned to one plant per container after germination. After planting, 300 post-chill WCR eggs were selected for uniformity and placed 5.08 to 7.62 cm deep in the soil near the seed in each container. In early July, fine nylon screen was wrapped securely around the upper rim of each bucket using elastic, and the free upper edge of the screen was securely tied around the corn stalk forming a trap for emerging adult beetles (Figures 5 and 6). Numbers of adults emerging per plant were recorded every three days until August 31, 1981.

Damage to corn roots was determined for all six locations by digging, washing, and examining roots for larval feeding damage. All roots were evaluated utilizing the 1-6 rating system used by the North Central States as the measure of insecticidal effectiveness. The rating criteria are as follows:

Damage Rating	Description of Root System
1	No noticeable feeding damage
2	Feeding scars, no root pruning
3	At least one root pruned but less than an entire node of roots pruned
4	One node of roots destroyed
5	Two nodes of roots destroyed
6	Three or more nodes of roots destroyed

To qualify as a pruned root, the root must be eaten to within 3.4 cm of the plant. It is not necessary for all pruned roots to originate from the same node to qualify as a root system with a full node pruned. The number of roots pruned must be equivalent to that of a full node.

Figure 5. WCR adult emergence trap as attached to pails' rim and corn plant.

Figure 6. WCR adult emergence traps showing field arrangement in artificial infestation.

Ten roots were tagged and evaluated from each replicate of each treatment at locations 1-5. At location six all roots were rated after adult emergence had subsided.

Data generated from soil test results, root damage ratings, and adult emergence were analyzed according to a randomized complete block design. Tests to detect differences between treatment means included Duncan's Multiple Range, LSD, and orthogonal comparisons.

RESULTS AND DISCUSSION

Laboratory Experiments

A preliminary laboratory experiment showed that aqua ammonia caused 100 percent mortality of WCR eggs at rates of 89 and 267 kg of nitrogen per ha. Expected survival of untreated eggs was 85%. These data indicate that aqua ammonia was successful in inhibiting hatch when applied directly to the eggs. Similar results were obtained by Hogan (1962) when gaseous ammonia was applied to diapausing eggs of *Acheta commodus* (Walk.), (Orthoptera: Gryllidae). Absorption of ammonia by the eggs accelerated the rate of termination of diapause but mortality occurred in all the ammonia treatments and increased with the severity of the experiment.

The second laboratory study involved application of aqua ammonia to WCR eggs in soil filled petri dishes. Expected survival of untreated eggs was 74% as determined by an untreated duplicate. Chi-square analysis was employed to compare observed egg mortality with expected egg mortality from ammonia treatments (Appendix A). Data indicate (Table 3) that observed egg mortality was greater than the calculated egg mortality due to the addition of aqua ammonia. A mortality curve (Figure 7) showed a decrease in rate response above the 712 kg/ha rate. $LSD_{0.05}$ was employed to detect differences in mortality between ammonia treatment means. Treatments 2, 5, and 6 had significantly more dead eggs than treatments 1, 4, and 5, respectively. These data indicate that the addition of aqua ammonia increased mortality and increasing the rate of applied nitrogen brought about a slower increase in mortality.

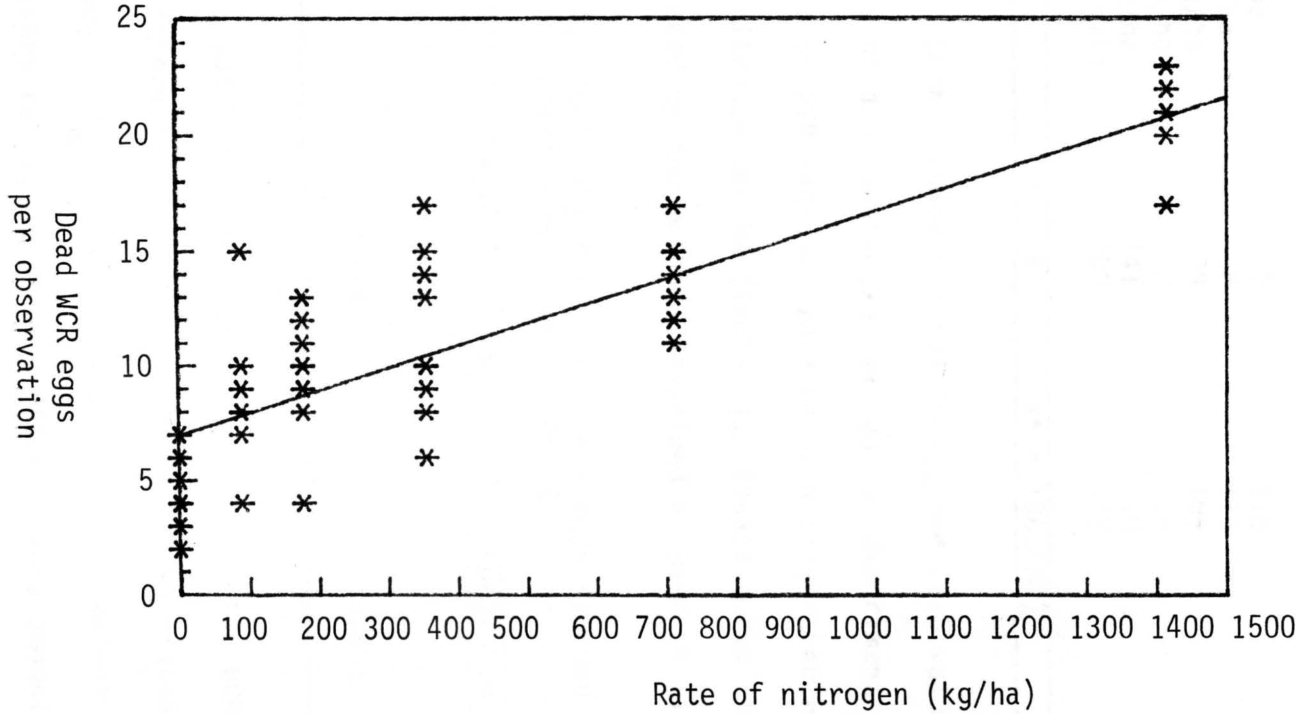

Figure 7. Mortality of WCR eggs in soil with various levels of aqua ammonia. ($y = 6.9775 + .0098X$. $r^2 = .748289$).

Table 3. Goodness of fit of X^2 of dead WCR eggs in experiment 2.

	Dead		Alive	
Classes	Observed Number	Calculated Number	Observed Number	Caluclated Number
0 kg/ha	48	118	239	169
89 kg/ha	88	115	190	163
178 kg/ha	99	105	157	151
356 kg/ha	112	119	177	170
712 kg/ha	141	115	139	141
1415 kg/ha	203	118	84	169
	df = 5	x^2 = 196.6873	P > .01	

Lethal concentration $(LC)_{50}$ and LC_{90} values were calculated for numbers of dead post-diapause eggs in experiment 2. The LC_{50} for aqua ammonia on WCR eggs was 1012 kg of nitrogen per ha. The LC_{90} was 27519 kg of nitrogen per ha (Table 4). Dosage mortality responses were calculated by the methods described by Daum et al. (1962).

Table 4. Mortality response of WCR eggs exposed to aqua ammonia for seven days in experiment 2.

	Dose (kg/ha N)	Lower Limit	Upper Limit
LC_{50}	1012	784	1355
LC_{90}	27519	13231	88402

Soil from petri dishes not containing WCR eggs were sampled at daily intervals for seven days. In the untreated check, water soluble nitrogen increased (Appendix G). Nitrate values remained constant in the dishes receiving various rates of aqua ammonia indicating that populations of nitrifying bacteria were inactive. Bagchi et al. (1977)

reported that numbers of nitrifying bacteria decreased with contact with ammonia gas in field experiments in India.

Application of ammonia to soil results in an initial increase in pH from the formation of ammonium hydroxide (NH_4OH) and a final decrease in pH when ammonium hydroxide forms ammonium ions (NH_4^+) and hydroxyl ions (OH^-). The initial soil pH was 7.5 and treatments increased at day one (Appendix H). Soil was retained for seven days only and there was not sufficient time for the pH to decrease to its original level. The highest rate of aqua ammonia (1415 kg/ha) caused the soil pH to increase to 8.5 compared to the untreated of 7.6 to 7.7 pH. In this experiment, extremely high rates of aqua ammonia were necessary to increase WCR egg mortality. Visual examination of the eggs after seven days was used to determine egg survival. Visual examination is an inexact method for determining egg viability (Jan Jackson, personal communication). Low mortality may have been due to dead eggs categorized as live eggs. Despite apparent low mortality in response to applied aqua ammonia, increasing the rate of nitrogen resulted in greater numbers of dead WCR eggs.

The final laboratory experiment involved determining the mortality of WCR eggs in 15.24 cm clay pots treated with aqua ammonia. Corn was planted in each as food for hatching larvae. Using the hypothesis that aqua ammonia increases WCR egg mortality, chi-square analysis was employed to compare observed mortality with calculated mortality when treated with various rates of aqua ammonia (Table 5). The addition of 89 kg of nitrogen per ha or higher resulted in a significant decrease in survival of WCR eggs compared to survival in the untreated check (Appendix B).

Table 5. Goodness of fit of X^2 of dead WCR larvae in experiment 3.

	Alive		Dead	
Classes	Observed Number	Calculated Number	Observed Number	Caluclated Number
0 kg/ha	217	271	83	29
89 kg/ha	245	271	55	29
178 kg/ha	269	271	31	29
267 kg/ha	277	271	23	29
356 kg/ha	294	271	6	29
712 kg/ha	298	271	2	29
1415 kg/ha	300	271	0	29
	df = 6	x^2 = 218.7681	P > .01	

Orthogonal comparisons showed a significant increase in egg mortality in response to fertilization except when comparing the mortality response of 356 kg/ha to 712 kg/ha, which was non-significant.

LC_{50} and LC_{90} values were calculated to roughly determine quantities of ammonia that could be used in field studies. The LC_{50} for aqua ammonia on WCR eggs and larvae is 116 kg/ha and the LC_{90} is 427 kg/ha. Upper and lower confidence limits are reported in Table 5.

Analysis of soil test values (Appendix C) show that both water soluble nitrates and pH values differ significantly between treatments. Nitrate values increase with increasing rates of aqua ammonia with the highest corresponding with 712 kg/ha (Appendix G). Increasing rates of aqua ammonia above 89 kg/ha resulted in a decrease in pH except for 1412 kg/ha which had a higher pH (Appendix H). Soil ammonia increased at rates of aqua ammonia above 356 kg/ha (Figure 8).

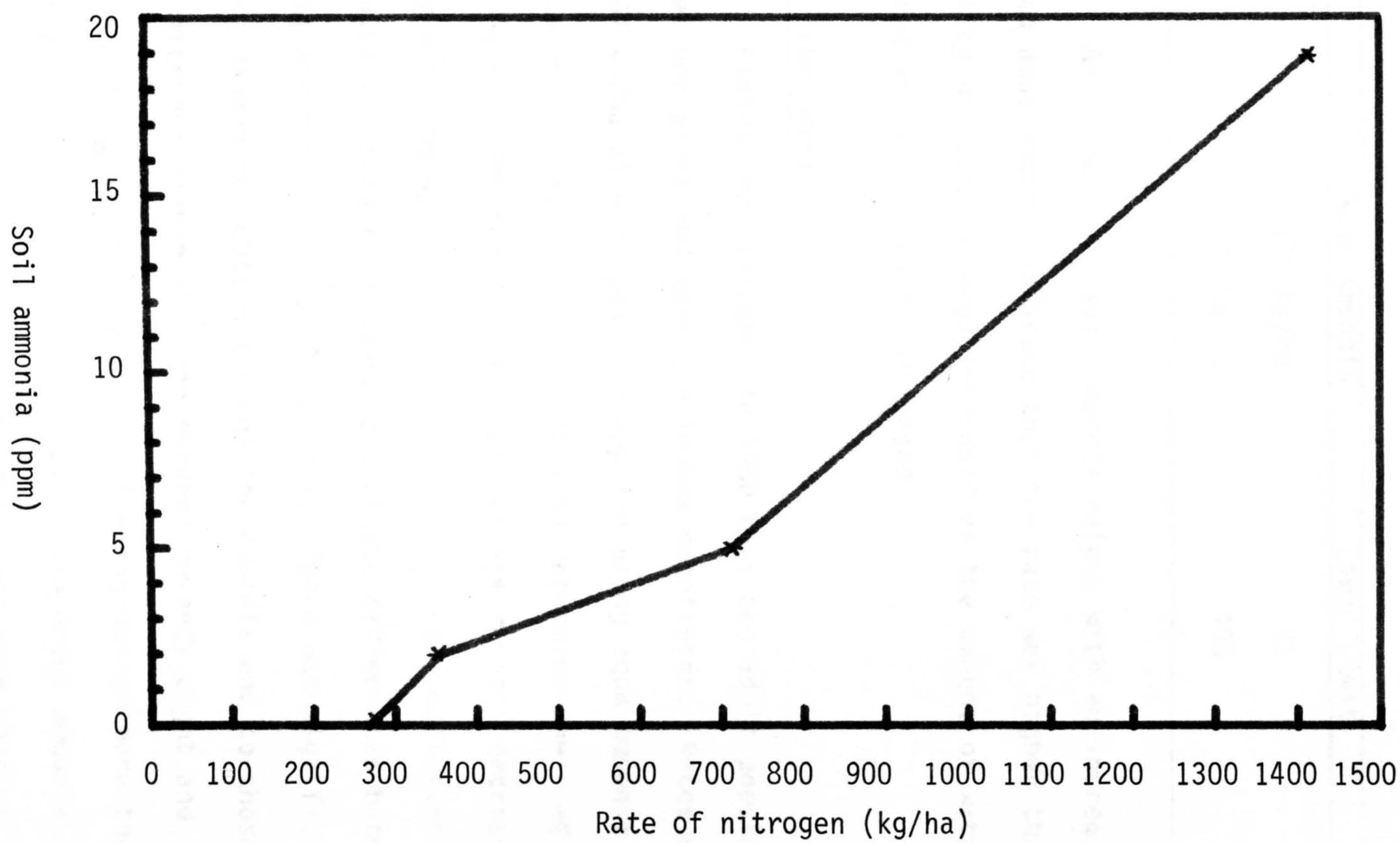

Figure 8. Rate of nitrogen applied as aqua ammonia vs soil ammonia content in experiment 3.

Table 6. Mortality response of WCR to increase in concentration of aqua ammonia.

	Aqua Ammonia	Lower Limit	Upper Limit
LC_{50}	116 kg/ha	41	183
LC_{90}	472 kg/ha	406	1155

An increase of soil ammonia values with an increase in rates of applied aqua ammonia indicate that the rate was higher than necessary. Mortality of rootworm eggs increased as the amount of water soluble nitrates and soil ammonia increased.

Field Experiments

Fields were selected in 1980 that had adult populations above 4 beetles per plant and were continuous cornfields. Rates of 0, 89, 178, and 267 kg/ha of nitrogen were applied using aqua ammonia and anhydrous ammonia on all five field locations to determine their effectiveness in reducing root feeding damage. Root damage ratings decreased significantly with the application of either nitrogen fertilizer (Appendix D). Duncan-Waller t-test was used to evaluate differences between levels of ammonia and between forms of ammonia. There was significantly more feeding damage to roots not receiving ammonia and to those receiving 89 kg/ha nitrogen (Table 7). One hundred seventy-eight and two hundred fifty-six kg/ha rates reduced root feeding damage more than the eighty-nine kg/ha treatment. The efficacy of anhydrous ammonia on reducing root damage was not significantly different than that of aqua ammonia. Ammonia was potentially effective as a control method in the field, but requires accurate placement over the previous year's row of corn, and an accurate placement of the planter over anhydrous ammonia. If not applied in this manner, a reduction in root feeding damage may not occur in the field because of rootworm egg distribution.

Table 7. Duncan-Waller t-test for root damage ratings.

Treatment	n	Mean	Grouping*	
Anhydrous				
1-UTC	20	2.3	A	
2-89 kg/ha	20	1.9	B	C
3-178 kg/ha	20	1.8	D	C
4-267 kg/ha	20	1.7	D	
Aqua				
5-89 kg/ha	20	2.0	B	
6-178 kg/ha	20	1.8	D	C
7-267 kg/ha	20	1.8	D	C

*Means followed by the same letter are not significantly different.

Soil samples were taken at each location one week prior to planting, one week after planting, three weeks after planting, and five weeks post-plant. First week post-plant data are not reported because improper drying may have altered soil test values. Third week post-plant soil pH increased significantly at all locations with increases in rates of applied ammonia. Rates of 89, 178, and 267 kg/ha all showed increased (more alkaline) pH values compared to the untreated check when soil samples were taken in the nitrogen application area. Water soluble nitrate nitrogen increased significantly with increases in rates of ammonia at locations 2, 3, and 4 only. Locations 3 and 4 reported significantly lower soil phosphorus values in plots receiving 267 kg/ha nitrogen. Plots receiving 267 kg/ha nitrogen had significantly higher soil ammonia values than plots receiving 0, 89, or 178 kg/ha nitrogen (Appendix E). Soil tests for nitrate, pH, phosphorus, and soil ammonia values had no detectable differences five weeks after planting. Plots receiving 89, 178, or 267 kg nitrogen per ha had the highest nitrate and ammonium soil test values with plots receiving 267 kg/ha having the highest soil ammonia concentration. Plots receiving nitrogen had significantly less root feeding damage than plots not receiving nitrogen although WCR population pressure was not high.

Field Artificial Infestation

Location 6 involved artificially infesting individual corn plants with 300 WCR eggs. Effects of aqua ammonia applications were measured by root damage ratings, and by the number of adult beetles emerging per treatment. Significantly less root damage was noted on roots receiving aqua ammonia treatments than unfertilized treatments. Both orthogonal comparison and LSD tests of differences between treatment means were not significant (Table 8) indicating that there was no rate response. Significantly more beetles emerged from corn not receiving ammonia than from plants receiving aqua ammonia (Table 9). LSD tests of differences between means indicate that the addition of ammonia resulted in a significant reduction in root damage ratings and significantly less adults emerging from traps.

Table 8. Aqua ammonia treatments vs. root damage rating in field artificial infestation.

Treatment	Total Rating	Mean Rating
1-0 kg/ha	37.2	3.1
2-89 kg/ha	30.0	2.5
3-178 kg/ha	28.8	2.4
4-267 kg/ha	19.2	1.6
Orthogonal Comparisons		
1 vs 2	--	n.s.
2 vs 3	--	n.s.
3 vs 4	--	n.s.

Table 9. Aqua ammonia treatments vs. numbers of adult WCR beetles emerging from soil.

Treatment	No. adults emerged per treatment	No adults emerged per plant
1-0 kg/ha	292	24.33
2-89 kg/ha	222	18.50
3-178 kg/ha	199	16.58
4-267 kg/ha	131	10.92
Comparisons		
1 vs 2	--	*
2 vs 3	--	n.s.
3 vs 4	--	*

*Significantly different at the $LSD_{.05}$ level.

Soil tests were taken in September 1981 after roots were examined for damage. Analysis of nitrates, pH, and soil phosphorus did not show significant differences in soil test values between fertilizer treatments.

Three hundred eggs per plant was sufficient for an average root rating of 3.1 in the untreated check. The addition of aqua ammonia reduced root damage ratings and numbers of adults emerging per plant. The emergence traps appeared to be very effective, and the design is worth considering for future studies.

CONCLUSIONS

1. A reduction in survival of WCR eggs and larvae occurred in aqua ammonia treated soil.
2. Field evaluations showed slight but significant reduction in root feeding damage with low rootworm population pressure. Additional studies are required under greater population pressure.
3. The influence of climatic conditions on nitrogen movement and rootworm egg mortality requires additional work.

APPENDIX

APPENDIX A

Analysis of variance of egg mortality in experiment 2

Source of Variation	d.f	SS	MS	Observed F	Required F .05	Required F .01
Treatment	5	130.2833	278.0567	40.0722**	2.38	3.37
Error	54	374.7000	6.9389			
Total	69	1764.9833				

**Significant at the $F_{.01}$ level

APPENDIX B

Analysis of variance of larval mortality in experiment 3

Source of Variation	d.f	SS	MS	Observed F	Required F .05	Required F .01
Treatment	6	528.6857	88.1143	9.2581**	2.24	3.09
Error	63	599.6000	9.5175			
Total	69	--	--			

**Significant at the $F_{.01}$ level

APPENDIX C

AOV of nitrate-nitrogen and pH values in experiment 3

Source of Variation	d.f	SS	MS	Observed F	Required F 5%	Required F 1%
	Nitrate-nitrogen					
Treatment	6	365545.1714	60924.1952	5.86**	2.24	3.09
Error	63	654712.1000	10392.2555	--		
Total	69	1020257.2714	--	--		
	pH					
Treatment	6	1.2214	0.2035	11.2**	2.24	3.09
Error	63	1.1530	0.0183	--		
Total	69	2.3744	--	--		

**Significant at the $F_{.01}$ level

APPENDIX D

Analysis of variance of fertilizer treatments vs root ratings at 5 field locations

Source	df	Anova SS	Observed F	Required F .05	Required F .01
Treatments	6	492.1000	8.703**	4.41	8.28
Location	4	76.1857	> 1.0		
Location X Rep	18	169.5000	1.52		

**Significant at the $F_{.01}$ level

APPENDIX E

Average root ratings at all field locations in 1981

Location	Treatment 1	2	3	4	5	6	7
1	2.3	2.3	1.9	2.0	2.0	2.0	2.0
2	2.3	1.9	1.8	1.8	2.1	1.7	1.7
3	2.4	1.8	1.8	1.6	2.0	2.0	1.6
4	2.3	2.0	1.9	1.7	2.0	1.7	2.0
5	2.6	1.9	1.8	1.7	2.0	1.8	2.0
6	3.1	2.5	2.4	1.6	---	---	---

Average soil pH after treatment at all locations in 1981 3 weeks after planting

Location	Treatment 1	2	3	4	5	6	7
1	6.2	6.2	6.2	6.2	6.2	6.1	6.2
2	6.3	6.3	6.3	6.2	6.4	6.4	6.4
3	6.3	6.3	6.2	6.4	6.4	6.3	6.5
4	6.6	6.7	6.4	6.5	6.6	6.5	6.5
5	5.6	5.8	5.6	5.6	5.5	5.6	5.6
6	6.7	6.7	6.7	6.6	---	---	---

Average water soluble nitrate at all locations in 1981 (kg/ha) 3 weeks after planting

Location	Treatment 1	2	3	4	5	6	7
1	64.4	69.7	62.3	56.8	65.9	68.1	87.1
2	66.2	53.9	49.7	71.3	58.8	45.0	61.5
3	44.8	37.7	61.9	39.9	42.1	37.2	42.8
4	59.5	77.0	65.3	74.2	59.1	74.6	68.4
5	63.2	64.4	75.1	73.9	66.4	84.2	83.1
6	9.5	9.1	10.9	9.6	--	--	--

APPENDIX E (Continued)

Average phosphorus soil test for all field locations in 1981 (kg/ha) 3 weeks after planting

Location	Treatment 1	2	3	4	5	6	7
1	29.4	28.8	28.9	32.3	30.3	28.5	30.7
2	22.1	26.3	25.7	30.3	24.9	22.5	24.9
3	22.7	20.3	20.5	18.3	26.3	18.5	18.1
4	27.9	28.1	24.8	24.3	27.0	33.0	23.9
5	52.6	52.4	54.6	54.2	61.0	59.0	60.1
6	10.9	11.4	11.0	11.0	--	--	--

Average soil ammonia values for all field locations in 1981 (ppm) 3 weeks after planting

Location	Treatment 1	2	3	4	5	6	7
1	66.5	64.3	68.7	66.4	83.7	107.6	98.8
2	98.4	92.5	79.3	90.0	90.2	85.0	93.2
3	102.8	102.5	118.3	102.7	108.8	125.9	129.8
4	96.1	96.7	103.4	88.0	89.2	106.4	98.1
5	88	95.9	110.0	103.4	112.5	151.2	153.2
6	--	--	--	--	--	--	--

APPENDIX F

Analysis of variance of root damage
ratings in field artifical infestation

Source	d.f	SS	MS	Observed F	Required F .05	.01
Replicates	3	.9167	.3056	.4632	3.49	5.95
Treatments	3	12.4167	4.1389	6.2739**		
Exp. Error	12	7.9166	.6597	--		
Sampling Error	32	28.0001	.8750	--		
Total	50	35.9167	--	--		

**Significant at the $F_{.01}$ level

APPENDIX G

Water soluble nitrate quantities over a seven-day period in experiment 2

Days	1	2	3	4	5	6	7
0 kg/ha	112	104	168	178	210	269	249
89 kg/ha	136	138	125	140	144	136	134
178 kg/ha	125	133	128	146	133	138	133
356 kg/ha	143	133	140	148	132	138	133
712 kg/ha	132	151	130	146	141	141	134
1415 kg/ha	162	148	140	136	156	104	117

APPENDIX H

PH over a seven-day period in experiment 2

Days	1	2	3	4	5	6	7
0 kg/ha	7.6	7.5	7.7	7.5	7.5	7.2	7.2
89 kg/ha	8.3	8.2	8.2	8.1	8.5	8.4	8.4
178 kg/ha	8.3	8.1	8.2	8.1	8.4	8.3	8.4
356 kg/ha	8.2	8.1	8.2	8.0	8.4	8.0	8.4
712 kg/ha	8.2	8.0	8.0	8.1	8.4	8.4	8.4
1415 kg/ha	8.1	8.1	7.9	8.1	8.5	8.4	8.5

APPENDIX I

Total numbers of dead WCR eggs at each treatment level of aqua ammonia in experiment 2

0 kg/ha		89 kg/ha		178 kg/ha	
Replicate	Dead eggs	Replicate	Dead eggs	Replicate	Dead eggs
1	4	1	8	1	13
2	3	2	7	2	8
3	6	3	10	3	10
4	7	4	4	4	12
5	5	5	8	5	11
6	3	6	15	6	13
7	3	7	9	7	9
8	4	8	10	8	10
9	5	9	8	9	9
10	4	10	9	10	4

356 kg/ha		712 kg/ha		1415 kg/ha	
Replicate	Dead eggs	Replicate	Dead eggs	Replicate	Dead eggs
1	17	1	17	1	16
2	10	2	17	2	17
3	13	3	13	3	19
4	9	4	12	4	19
5	8	5	15	5	15
6	15	6	11	6	16
7	14	7	12	7	16
8	10	8	15	8	16
9	10	9	14	9	15
10	6	10	15	10	14

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